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Mechanisms of ß-lactam resistance among *Pseudomonas aeruginosa*

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Abstract
A total of 100 clinical isolates of *Pseudomonas aeruginosa* from inpatient and outpatient were isolated from various clinical samples from January 2021 to December 2021 in a tertiary care hospital in Bilaspur, Chhattisgarh. Uropathogenic *P. aeruginosa* infections were more prevalent in females than in males. Ciprofloxacin, Piperacillin, Imipenem were found more effective for treatment of infections in outpatients but for inpatients, parental therapy with newer aminoglycosides and third and fourth generation cephalosporins were found to be effective. About 99% of the clinical isolates were resistant to six commonly used antibiotics- ampicillin (100%) and cefuroxime (100%), amoxicillin (99%), co-trimoxazole (99%), Tetracycline (99%), cefazoline (99%). *In vitro* sensitivity pattern of 100 isolates of *P. aeruginosa* showed hight sensitivity to imipenem (97%), amikacin (79%), tobramycin (70%), ceftazidime (62%), ciprofloxacin (73%), cefoperazone (60%), piperacillin (65%), gentamycin (34%) and cefotaxime (14%). ESBL producing strains (33%) were more resistant to beta-lactams and other antibiotics. The results indicate that *P. aeruginosa* is commonly responsible for the nosocomial infections in Jagdalpur.

Keywords: Antibiotics, cross-resistance, pseudomonas, susceptibility

Introduction
Pseudomonas aeruginosa is a versatile, innocuous, Gram negative, oxidase positive, motile bacteria commonly associated with nosocomial infections. In spite of the use of potent antibiotics, mortality is high in case of *P. aeruginosa* infections. Nosocomial multidrug resistant *P. aeruginosa* is an important health care problem worldwide that prolongs the duration of hospitalization, thereby increasing the cost of patient care. Considering the problem of *P. aeruginosa* infection and multi drug antibiotic resistance, the present study has been carried out to determine current trends of antibiotic resistance among *P. aeruginosa* strains causing various nosocomial and community acquired infections. *P. aeruginosa* is ubiquitous in the nature and can be isolated from soil and water (National Institute of Health, 1994) and occasionally from normal human skin (Percy DH *et al*. 1993) [16]. It can inhabit the nasopharynx, lower digestive tract (Weisbroth, 1979). However, carriage increases with the length of stay in hospital, reaching 30 - 50% after 3 weeks and thus can present a distinct risk of endogenous infection (Neu, 1983). *P. aeruginosa*, an increasingly prevalent opportunistic nosocomial pathogen. It can infect almost any external site, and therefore, can be isolated from various body fluids such as sputum, urine, wounds, eye or ear swabs and from blood (Hugbo and Olurinola, 1992). *P. aeruginosa* may develop several mechanisms of resistance against a variety of antibiotics. ESBL producing organism pose unique challenges to clinical microbiologists and clinicians. ESBLs are enzymes capable of hydrolyzing third and fourth generation cephalosporins such as ceftazidime, ceftoxime and cefepime as well as aztreonam. Currently carbapenems are regarded as the drug of choice for treatment of infections caused by ESBL-producing organisms.

Materials and Method
A total of 100 *P. aeruginosa* isolates from clinical samples of pus, urine, blood, different body fluids, throat, sputum and ear swabs, both from outdoor and indoor patients of different wards were aseptically collected during January 2021 to December 2021 submitted to Dept. of Microbiology, Institute of Medical Sciences, Bilaspur and identified on the basis of colony morphology according to Bergey's Manual of Determinative Bacteriology, 8th edition.

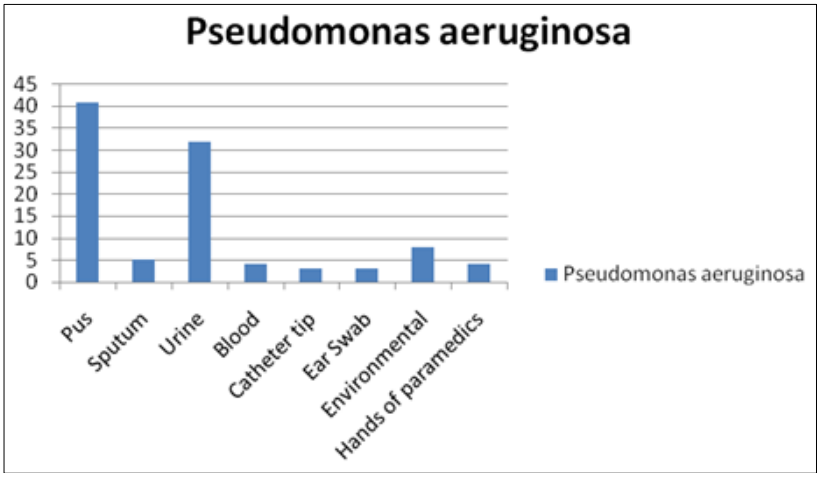
Antimicrobial susceptibility testing was carried out by disk diffusion method of Bauer *et al*. (1966) [2]. Antibiotic susceptibility testing for each bacterial isolate was done on Muller Hinton agar in a 90 mm sterile Petri dish and incubated at 37ºC for 18hrs. The panel of antimicrobials tested were ampicillin (10µg), amikacin (30 µg), amoxycillin (25µg), cefotaxime (30 µg), cefuroxime (30 µg), cefoperazone (75 µg), ciprofloxacin (5 µg), gentamycin (10 µg), imipenem (10 µg), piperacillin (100 µg) and Tetracycline (30 µg) (HiMedia). *P. aeruginosa* (ATCC27853) was used as the control strain. After incubation, plates were examined for zones of inhibition and categorized to sensitive, intermediate and resistant according to National Committee for Control Laboratory Standards (NCCLS, 1993). Double disc diffusion method was used to detect the extended spectrum beta lactamases (ESBL). A disk of co-amoxicillin (20 µg amoxicillin/10 µg clavulanic acid) was placed in the center of the agar surface. The discs of cephotaxime, ceftriaxone, ceftazidime and aztreonam (30 µg) were arranged in such a way that the distance between the central disc and surrounding discs was 20 mm. The plates were incubated at 37°C for 24 h. If the inhibition zone around one or more cephalosporins discs was extended on the side nearest to the co-amoxiclave disc, the organism is an ESBL - producer.

Results

A total of 100 isolates of *P. aeruginosa* isolated from various specimens (43 were isolated from males, 46 from females and 8 from environmental sources) are included in the present study. The samples comprised of pus, urine, blood, sputum, ear swabs and catheter tips and from the hands of paramedics (Table 1). Most isolates were from indoor patients (84%). Among clinical isolates, *P. aeruginosa* was most prevalent in pus (44%), followed by urine (34%), sputum (5%), blood (4%), catheter tips (3%) and ear swabs (3%). Among 41 isolates of pus, 23 (57%) were from males and 18 (43%) were from females. Among 32 isolates of urine, 18 (56%) were from females and 14 (44%) from males. Table II shows the susceptibility pattern of the 100 isolates. Among penicillin group (beta-lactam antibiotics), none of the isolates were found sensitive to ampicillin and no isolate showed intermediate behavior while rest of the isolates showed highly resistant behavior (100%). Piperacillin (Beta-lactamases inhibitor) was found effective against 65% isolates. Three amino-glycosides i.e. tobramycin, amikacin, and gentamycin showed 79%, 70% and 34% sensitivity respectively. Among the quinolones and fluoroquinolones, ciprofloxacin was found effective against 73% isolates. Resistance to co-trimoxazole was 100%. Among 3rd generation cephalosporins, ceftazidime and cefotaxime showed 62% and 14% sensitivity respectively. Tetracycline was found effective against 1% of the isolates. Cefoperazone (extended-spectrum cephalosporin) inhibited 60% of the isolates, 3% isolates showed intermediate behavior, while, 37% isolates were resistant. Imipenem (carbapenems) was found to be the most effective antibiotic in this study, 97%. Isolates were sensitive. Isolates exhibited 100% resistance to cefuroxime, amoxilillin and ampicillin. Isolates were highly resistant to (99%) tetracycline, cefazoline, co-trimoxazole, 86% to cefotaxime, 66% to gentamycin, 38% to Ceftazidime, 37% to cefoperazone, 32% to piperacillin, 30% to tobramycin, 26% to ciprofloxacin, 21% to amikacin, and 3% to Imipenem (Table-II). The present study was conducted to determine the prevalence, resistance and phenotypic transfer of ESBLs among *P. aeruginosa* isolates. Out of 100 isolates, 33 (33%) were found to be ESBL producers. Fourteen (31.8%) isolates from pus and 11 (34.3%) from urine were found to be ESBL producers.

Table 1: Isolation sources of *Pseudomonas aeruginosa*

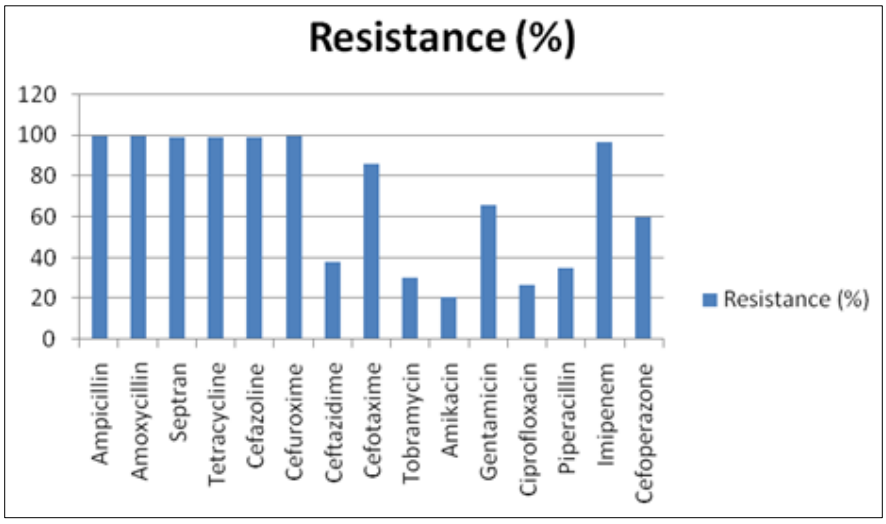
Specimen	*Pseudomonas aeruginosa*
Pus	41
Sputum	5
Urine	32
Blood	4
Catheter tip	3
Ear Swab	3
Environmental	8
Hands of paramedics	4
Total	100



Graph 1: Isolation sources of *Pseudomonas aeruginosa*

Table 2: Sensitivity pattern of *Pseudomonas aeruginosa*

Antibiotics	Resistance (%)
Ampicillin	100
Amoxycillin	100
Septran	99
Tetracycline	99
Cefazoline	99
Cefuroxime	100
Ceftazidime	38
Cefotaxime	86
Tobramycin	30
Amikacin	21
Gentamicin	66
Ciprofloxacin	27
Piperacillin	35
Imipenem	97
Cefoperazone	60



Graph 2: Sensitivity pattern of *Pseudomonas aeruginosa*

Discussion

A total of 100 *P. aeruginosa* isolates from clinical samples of pus, urine, blood, different body fluids, sputum and ear swabs, from outdoor as well as indoor patients, Similar pattern of isolation of *P. aeruginosa* from pus, urine, ear, nose, wound and other infection sites was reported earlier (Ergin and Mutlu, 1999) [7], but in a study conducted by Olayinka (Olayinka *et al*., 2004) [15], *P. aeruginosa* was mostly isolated from urine samples. This could be attributed to differences in geographical location and hygienic measures or to the fact that most patients going for major surgery tend to get catheterized. In the present study, uro-pathogenic *P. aeruginosa* was found higher in females than males. Garibaldi *et al.* have also documented a higher risk for developing bacteriuria in adult female patients, the elderly and critically ill patients with a urinary catheter. Longer stay in the hospital increases the colonization of skin and environment of the patient and may be responsible for higher incidence of urinary catheter related infections (Tullu *et al*., 1998) [20]. However *P. aeruginosa* is said to be responsible for pneumonia and septicaemia with attributable deaths reaching 30% in immunocompromised patients (Fergie *et al*., 1994; Dunn and Wunderink, 1995; Brewer 1996) [8, 6, 5]. This is in agreement with Cruse (1973) who reported that the hands of nurses working in wards with infected patients often carry *P. aeruginosa*. Similar kind of results was reported by others (Oguntibeju and Nwobu, 2004) [14]. *P. aeruginosa* is currently one of the most frequent nosocomial pathogen and the infections due to this organism are often difficult to treat due to antibiotic resistance (Emori and Gaynes, 1993). The mechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal and plasmid mediated beta-lactamases (Livermore, 1989) [13], aminoglycoside-modifying enzymes (Livermore, 1987) [12] and an active multidrug efflux mechanism (Li, 1994; Shahid and Malik, 2004) [11, 17]. In the present study, *P. aeruginosa* showed high resistance to commonly used antimicrobials limiting therapeutical options. Other studies (Wise *et al*., 1979; Karmali *et al*., 1980) [10] also showed similar findings. During the study, we observed that the alginate capsules of mucoid strains of *P. aeruginosa* could not act as a barrier against imipenem. This finding is comparable to the results of Slack and Nichol's studies, in which alginate impeded the penetration of all antibiotics except the beta-lactams (Slack and Nichols, 1981; Rezaee *et al*., 2002) [18]. The development of antimicrobial resistance is an evolutionary process, driven by selective pressure. Nosocomial multidrug resistant *P. aeruginosa* is an indolent health care problem worldwide despite stringent hospital antimicrobial policies in place. Decreasing unnecessary antibiotic use, treating with narrow spectrum agents, improving compliance with therapy, decreasing use of antibiotic in animal and agriculture, and improving infection control all have a role in confronting this problem.

References

1. Al-Lawati AM, Crounch ND, Elhag KM. Antibiotic consumption and development of resistance among gram-negative bacilli in intensive care units in Oman. Annal. Saudi Med. 2000;20:324-327.
2. Bauer AW, Kirby WM, Sherris JC, Truck M. Antibiotic susceptibility testing by standardized single disc method. Am. J Clin. Path. 1966;45:493-496.
3. Bonfiglio G, Laksai Y, Franchino L. Mechanisms of ß-lactam resistance amongst *P. aeruginosa* isolated in an Italian survey. J. Antimicrob. Chemother. 1998;42:697-702.
4. Botzenhart K, Doring G. Ecology and epidemiology of *Pseudomonas aeruginosa* IN: Campa, M, eds *Pseudomonas aeruginosa* as an Opportunistic Infection New York: Plenum Press, 1993, 1-18.
5. Brewer SC, Wunderink RG, Jones CB, Leeper KVJ. Ventilator associated pneumonia due to *P. Aeruginosa*. 1996;109:1019-1029.
6. Dunn M, Wunderink RG. Ventilator-associated pneumonia caused by pseudomonas infection (Review) Clinics in Chest Med. 1995;16:95-109.
7. Ergin C, Mutlu G. Clinical distribution and antibiotic resistance of Pseudomonas Species. Eastern J Med. 1999;4(2):65-69.
8. Fergie JE, Shama SJ, Lott L, Crawford R, Patrick CCP. *P. aeruginosa* bacteraemia in immuno-compromised children: analysis of factors associated with a poor outcome. Clin. Infect. Dis. 1994;18:390-394.
9. Garibaldi RA, Burke JP, Dickman ML, Smith CB. Factors predisposing to bacteriuria during indwelling urethral catheterization. New Engl. J Med. 1974;291:215-219.
10. Karmali MA, De-Grandis S, Fleming PC. Antimicrobial susceptibility of campylobacter jejuni and campylobacter fetus subsp Fetus to eight cephalosporin's with special resference to species differentiation. Antimicrob. Agent Chemother. 1980;18(6):948-951.
11. Li XZ, Livermore DM, Nikaido H. Role of efflux pump(s) in intrinsic resistance of *P. aeruginosa*: resistance to tetracycline, chloramphenicol and norfloxacin. Antimicrob. Agents Chemother. 1994;38(8):1732-1741.
12. Livermore DM. Clinical significance of beta-lactamase induction and stable derepression in gram-negative rods. Eur. J Clin. Microbiol. 1987;6:439-445.
13. Livermore DM. Role of Beta-lactamase and impermeability in the resistance of *P. aeruginosa*. Antibiot. Chemother. 1989;42:257-263.
14. Oguntibeju OO, Nwobu RAU. Occurrence of *pseudomonas aeruginosa* in postoperative wound infection. Pak. J Med. Sci. 2004;20(3):187-191.
15. Olayinka AT, Onile BA, Olayinka BO. Prevalence of multi-drug resistant (mdr) pseudomonas aeruginosa isolates in surgical units of ahmadu bello university teaching hospital, zaria, nigeria: an indication for effective control measures. Annal. Afri. Med. 2004;3(1):13-16.
16. Percy DH, Barthold SW. Pathology of Laboratory Rodents and Rabbits. 1993;1:37-38, 2:85-86.
17. Shahid M, Malik A. Plasmid mediated amikacin resistance in clinical isolates of *Pseudomonas Aeruginosa*. Ind. J Med. Microbiol. 2004;22(3):182-184.
18. Slack MP, Nichols WW. The penetration of antibiotics through sodium alginate and through the exopolysaccharide of a mucoid strain of *P. aeuroginosa*. 1981;2:502-503.
19. Thornton GF, Andriole VT. Bacteriuria during indwelling catheter drainage Effect of closed sterile drainage system JAMA. 1970;214:339-342.
20. Tullu MS, Deshmukh CT, Baveja SM. Bacterial profile and antimicrobial susceptibility pattern in catheter related nosocomial infections JPGM. 1998;44(1):7-13.
21. Wise R. Beta-Lactams; Cephalosporins in Antibiotics and Chemotherapy, 7th edn, O'Grady F, Lambert PH, Finch RG, 1997.